Cytochrome P450 1A Enzymes As Non-Invasive Biomarkers of Contaminant Exposure in Skin from Harbour Seals *(Phoca vitulina)*

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The marine environment is contaminated with persistent chemicals including PCBs (polychlorinated biphenyls), PCDDs (polychlorinated-dibenzo-p-dioxins) and PCDF (polychlorinated-dibenzo-p-furans). These lipophilic compounds tend to biomagnify in the food chain reaching high levels in marine mammals, including harbour seals. The cytochrome P450 1A subfamily has been used as biomarker of contaminant exposure as a result of their induction in wildlife (fish, birds, mammals) that inhabit industrialized areas. By determining the CYP 1A1/2 content (western blotting) and its associated enzyme activities (EROD and MROD), we are developing a minimal-invasive method by using this biomarker in skin biopsy samples collected from free-ranging harbour seals.

Skin/blubber biopsies were collected from 20 young seals , aged approximately 4 to 6 weeks, from the Fraser estuary near Vancouver, British Columbia. Two induction studies using b-naphthoflavone (BNF), a non-toxic inducer of CYP1A enzymes, were also carried out in two groups of six seals each as a means of characterizing the responsiveness of these enzymes using both in vivo oral (50 mg/kg body weight) and topical (0.12mg BNF/cm2) administration.

This non-destructive biomarker approach may provide us with an important tool for understanding the risks associated with contaminant exposure in different marine mammals, including those from which no liver samples can be obtained (e.g. killer whales).

An Ecosystem Approach to Assessing the Accumulation of Persistent Organic Pollutants (POPs) in the Food Chain of Harbour Seals *(Phoca vitulina)* Inhabiting the Coastal Waters of British Columbia And Washington

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There is increasing concern for the ecological and human health risks of persistent organic pollutants (POPs). These contaminants have been found in higher trophic level organisms including marine mammals. Exposure to POPs has been associated with adverse health effects in marine mammals including immunotoxicity and endocrine disruption. Recent research has found high PCB concentrations as well as a contaminant-related disruption of retinoid physiology in B.C. and Washington harbour seals (*Phoca vitulina*). Tools and techniques including congener-specific contaminant analyses, fatty acid signature analyses, stable isotope ratios, and multivariate statistical analyses will enable us to characterize the bioaccumulation of POPs in the harbour seal food chain (eg. herring, hake, salmonids). Understanding the origin of these POPs in harbour seals is hampered by the complexity of transport and fate processes, which

reflect a combination of both local and global (eg. atmospheric deposition) sources. We are developing a contaminant-based model to describe bioaccumulation in the food chain of harbour seals inhabiting Puget Sound and the Strait of Georgia. This research will help to elucidate pathways of POPs in Puget Sound and the Strait of Georgia using harbour seals as sentinels of marine ecosystem contamination.

Should We Be Concerned About Estrogenic Compounds in Puget Sound Waterways? Adult Male English Sole Collected Near Urban Areas May Provide the Answer

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Vitellogenin (Vtg) is a yolk protein produced in the liver of oviparous animals in response to estrogen. Its synthesis is normally observed only in sexually mature females with developing eggs, however male animals can synthesize Vtg when exposed to exogenous estradiol or to substances that mimic estrogens. Thus, the abnormal production of Vtg in male animals can be used as a biological indicator for exposure to environmental estrogens. As part of the Puget Sound Ambient Monitoring Project (PSAMP), we collected plasma from adult male English sole from numerous urban embayments in Puget Sound between 1997-99. Among the sites sampled are areas with elevated levels of compounds in bottom sediments with suspected estrogenic activity, such as certain phthalates, DDTs, and PCB congeners (Sinclair Inlet, Duwamish Waterway, Elliott Bay). Male English sole plasma from these sites and several clean reference sites are being analyzed utilizing a validated enzyme-linked immunoassay (ELISA) for plasmatic Vtg in English sole. The presence or absence of Vtg in these samples will help to answer the above question. The results of this study will be presented and the implications discussed.

Cytochrome P450 Enzymes: Biomarkers of Contaminant Exposure in English Sole and Harbour Seals in Southern British Columbia

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We are assessing the use of the enzyme cytochrome P450 1A (CYP1A) as a biomarker for contaminant exposure in fish and marine mammals in southern British Columbia. Hepatic ethoxyresorufin-O-deethylase (EROD) activity and CYP1A levels were measured in English sole caught at three industrial sites and two reference sites in and around Vancouver Harbour. We found that fish with high EROD activity and CYP1A levels came from sites containing relatively high sediment levels of polyaromatic hydrocarbons and organochlorines. There also appeared to be positive correlations between EROD and CYP1A results, liver lesions, and bile levels of low molecular weight aromatic hydrocarbons. In a separate study, we collected

liver biopsies from 16 young harbour seals. Seals were live-captured from the Fraser River estuary near Vancouver, British Columbia and temporarily housed in captivity. Liver biopsies were taken under general anaesthetic. We will measure EROD activity and CYP1A1/1A2 levels in these liver samples, and these results will be compared to enzyme levels detected in other physiological compartments, as well as to other toxicological endpoints. This research will enable us to assess the relative importance of local contamination (English sole) and ecosystem or food chain contamination (harbour seals).

Vitamin A as a Biomarker of Contaminant Toxicity in Harbour Seals

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Vitamin A is a collective name for a group of small fat-soluble molecules (also called retinoids) that are essential to all mammals in processes of growth and development, as well as for the maintenance of reproductive, endocrine and immune systems. Although its levels are highly regulated within the body, environmental contaminants, such as polychlorinated and -brominated compounds (e.g. PCBs), can disrupt the homeostasis of this dietary hormone. Vitamin A therefore serves as a biomarker of contaminant exposure and effect. Recently, our laboratory documented a contaminant-related increase in circulatory vitamin A in free-ranging harbour seals sampled in British Columbia and Washington. As part of our ongoing ecotoxicology research, we live-captured and temporarily housed 20 healthy, recently weaned harbour seal (*Phoca vitulina*) pups from British Columbia's coast. Blood and micro-scale tissue samples (skin, blubber and liver biopsies) obtained during this semi-field study were quantified for different retinoids. This compartment model of vitamin A homeostasis in harbour seals will i) document mechanisms of action which would explain the observed vitamin A disruption in seals; and ii) serve to validate novel means to assess contaminant-related toxicity in species where blood sampling is not possible (e.g. killer whales).

Temporal Trends in Toxicopathic Hepatic Lesion Occurrence in English Sole (*Pleuronectes vetulus*) from Puget Sound, Washington: Results of the Puget Sound Ambient Monitoring Program; 1989-1999

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The Washington State Department of Fish and, as part of the Puget Sound Ambient Monitoring Program has monitored the occurrence of toxicopathic liver lesions and contaminant levels in muscle and liver tissue in English sole *(Pleuronectes vetulus)* at more than 40 sites in Puget Sound. Lesion prevalence in English sole is monitored as a general indicator of contaminant-related fish health because research by the National Marine Fisheries Service and Fish and Wildlife has shown that although the risk of developing liver lesions increases with fish age, exposure to contaminated sediments particularly high molecular weight PAHs, is the main risk factor associated with developing lesions. Furthermore, reproductive impairment has also been observed in English sole at sampling sites with elevated occurrences of liver lesions.

Six sites were monitored annually between 1989 and 1999 to track temporal changes in lesion occurrence and included two non-urban (Strait of Georgia and Hood Canal), one near-urban (Port Gardner) and three

urban locations (Elliott Bay, Sinclair Inlet and Commencement Bay). Average lesion occurrence in English sole was highest at Elliott Bay and Commencement, intermediate at Sinclair Inlet and Port Gardner and lowest at Strait of Georgia and Hood Canal. As compared to the risk of liver lesion occurrence at 19 other non-urban, relatively uncontaminated reference sites (defined as 1.0), the risk of developing liver lesions apparently increased at Elliott Bay between 1989 and 1999 but not at other sites. Possible factors affecting the increase in liver disease in English sole from Elliott Bay are presented.